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Agriculture Development Fund

FINAL REPORT

19970298

RESPONSE OF HISTORICAL AND CURRENT WESTERN CANADIAN SPRIING WHEAT TO SYMBIOSIS WITH MYCORRHIZAL FUNGI

Funded by
The Agriculture Development Fund

February 2002

Prepared by: University of Saskatchewan

SAF
Saskatchewan Agriculture & Food
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Project Number: 97000298

Project Title: Response of Historical and Current Western Canadian Spring Wheat Cultivars to Symbiosis with Mycorrhizal Fungi

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Other Sponsors: Western Grains Research Foundation

Final Report: November 30, 2001

(a) ABSTRACT/SUMMARY:

Arbuscular mycorrhizal fungi (AMF) form symbioses with most terrestrial plants, including wheat.. This investigation assessed the interaction of AMF in Saskatchewan soils with modern (CDC Teal) and historic (PI 167549, Red Fife) wheat cultivars. The AMF spore population in the soil surrounding the roots of modern and historic wheat cultivars at two field sites was examined in 1998 and 1999, and more AMF spores were found associated with the historic cultivars. Previous cropping effects on AMF symbioses with modern and historic wheat cultivars were examined in the 2000 field season. Root colonization of wheat was highest on wheat stubble soil, intermediate on fallow soil and was lowest on canola stubble soil, indicating that previous crop affected the community structure as well as the population size of AMF in soil. The effect of soil phosphorus availability on AMF symbiosis with modern and historical wheat was evaluated in a growth chamber experiment. At low P availability, historic cultivars had a higher level of AMF root colonization, whereas at high P availability the modern cultivar was more extensively colonized. This demonstrated that AMF associated with historic cultivars had the potential to confer greater benefit to the host plant through increased nutrient uptake. However, the tissue P concentration and P uptake efficiency of the modern cultivar was higher, indicating less dependence on AMF for P nutrition. This research demonstrated differences in the way that modern and historic wheat cultivars interact with AMF in Saskatchewan soils, however environmental conditions were also important to the formation of a successful symbiosis.

(b) EXECUTIVE SUMMARY:

Arbuscular mycorrhizal fungi (AMF) form an obligate association with most plant roots and can influence crop growth, nutrient uptake and yields. The main benefit of AMF is their ability to stimulate plant growth in soils with limited amounts of available P, where the AMF external mycelium provides a larger surface area for increased absorption of P. However, there is a growing body of evidence that plant genotype controls the ability of the plant to be colonized by AMF and to respond positively to this symbiosis. Our recent studies have focused on how wheat cultivars respond to AMF under a variety of field and growth chamber conditions. The objective of this study was to determine if wheat breeding practices have reduced the ability of modern Canadian spring wheat cultivars to participate in, and respond to, mycorrhizal symbiosis.

Three wheat cultivars were chosen for extensive study throughout the research project. The first cultivar, PI 167549 is a historic Turkish cultivar and the second, Red Fife is a historic western Canadian cultivar registered in ca. 1841. Both of these historic cultivars were chosen because they represent cultivars that have not been subjected to improvement by crop breeders. The third cultivar, CDC Teal, is a modern spring wheat cultivar developed at the University of Saskatchewan Crop Development Center, registered in 1991.

A two-year field trial (1998, 1999) was conducted at the Crop Development Centre (CDC) Seed Farm (SF) and North Seed Farm (NSF) sites to evaluate the interaction of native arbuscular mycorrhizal fungi (AMF) in Saskatchewan field soils with historic and modern wheat cultivars. A second field trial was conducted in the 2000 crop year to evaluate the effect of previous crop on the interaction of AMF with historic and modern wheat cultivars. In addition, a growth chamber study was conducted to study the influence of soil phosphorus (P) availability on the ability of historic and modern wheat to exploit symbiosis with indigenous AMF from a Saskatchewan field soil. More specifically, a low P soil (<12mg/kg Olson P) was collected from a field near Conquest, Saskatchewan, mixed 1:1 with sterile silica sand and increments of 0, 15, 30 and 45 mg/kg phosphorus were added to corresponding pots. Each of the modern and historical cultivars were grown at the four P levels and plant growth and mycorrhizal parameters evaluated after 52 days of growth.

In 1998 an extensive evaluation of the arbuscular mycorrhizal fungal spore population at the North Seed Farm field site was conducted. The total number of AMF spores associate with the wheat cultivars was highest for PI 167549, intermediate for Red Fife and lowest for the modern cultivar CDC Teal. There also seemed to be differences in the number of AMF spore morphotypes associated with the three cultivars. Arbuscular mycorrhizal root colonization of the wheat cultivars in 1998 and 1999 showed that there was very little interaction between the AMF in the SF and NSF soils and historic and modern wheat cultivars (i.e. AMF colonized only 2-8% of total root length of the wheat cultivars). In 1999 a most probable number experiment found that no infective AMF propagules could be detected in either of the soils. Soil testing in the spring of 1999 demonstrated that the level of available soil phosphorus (P) was extremely high (>60

lb/ac). The inhibition of AMF symbiosis by high phosphorus availability may therefore have been the main factor in the lack of response of the wheat cultivars to AMF populations in the field soils.

In the 2000 field season, a field trial was conducted at the CDC Kernen Research Farm near Saskatoon, SK to evaluate the effect of cropping history on the AMF symbiosis with historic and modern wheat cultivars. Three field tests were set up on fallow, wheat stubble and canola stubble soils. An MPN of the three soils showed differences in the number of AMF propagules in the soil at the beginning of the growing season. Wheat stubble had the largest number of AMF, followed by fallow and lastly canola stubble. There were no significant differences in the AMF root colonization between the three cultivars, however colonization of the wheat roots was markedly different between fallow, wheat stubble and canola stubble soils. Wheat is a host crop for AMF and plants grown on the wheat stubble soil demonstrated the highest level of AMF root colonization. Fallowing of soil in 1999 decreased the number of AMF propagules and subsequently the level of AMF root colonization in wheat grown in 2000. Canola is a non-host crop for AMF and decreased the size of the AMF population in the soil. Subsequently, AMF root colonization was lowest in this soil. Sampling date also affected AMF root colonization of field grown wheat. Root colonization was significantly higher at 52 and 89 days after planting than after 36 days (Appendix B1).

In the growth chamber study, a more specific evaluation of phosphorus effects on modern and historic wheat cultivars and their ability to interact with AMF demonstrated that in fact, these cultivars interact with AMF differently under certain environmental conditions. Average shoot biomass production over all P levels was significantly higher for Red Fife and CDC Teal than for PI 167549. Conversely, root biomass production was higher for PI 167549 and Red Fife than for CDC Teal. Finally, the shoot:root ratio of CDC Teal was significantly higher than that of Red Fife and PI 167549. These results demonstrate inherent differences in the growth patterns of the three cultivars, with the historic PI 167549 producing more root biomass, relative to shoot biomass and the modern cultivar CDC Teal doing the opposite. The historic Red Fife cultivar shares the characteristic of large root biomass production with PI 167549. However, Red Fife was historically a large producer of shoot biomass and was taken out of production primarily because of improvements in disease resistance in newer cultivars. Phosphorus content of shoot material of these cultivars was also significantly different. CDC Teal took up the most P, Red Fife significantly less and PI 167549 the least. While these results indicate that CDC Teal has an inherent ability to take up more P, it should be noted that P content of the root material was not measured. However, allocation by the plant of P to shoot and seed production represents an increased level of P efficiency and it is therefore likely that the modern cultivar is more P efficient. Phosphorus availability of the soil was also important and the addition of 15 mg/kg P produced a significant increase in both shoot and root biomass. However, additions of P above this level did not result additional biomass production.

Arbuscular mycorrhizal root colonization, averaged over all P levels was significantly higher in CDC Teal than Red Fife and PI 167549. However there was a

significant interaction effect of cultivar and P level, indicating that AMF root colonization of each of the cultivars is dependent on the specific availability of phosphorus. In addition, each increasing P level caused a corresponding significant decrease in AMF root colonization.

Fatty acid methyl ester (FAME) analysis of root and soil material also demonstrated differences in the amount of the mycorrhizal FAME biomarker 16:1ω5cis, reported as an adjusted response ratio (ARR) of 16:1ω5*cis* to the total fatty acid content of the sample. The ARR of root material was significantly higher for CDC Teal than for PI 167549. When FAME analysis of the soil was conducted the ARR was significantly higher for PI 167549 and Red Fife than CDC Teal. These results indicate that there was more mycorrhizal growth within the root of the modern cultivar, and more external mycorrhizal growth in the historic cultivars. This would indicated that historic cultivars interact more efficiently with AMF, as primary growth is in the form of external hyphae that are able to aid the plant in nutrient acquisition.

Conclusions:

Modern and historic wheat cultivars did not interact differently with AMF in the field. In 1998 and 1999, extremely high soil phosphorus availability was likely the main factor in the inhibition of the formation of successful symbiosis. Repeated fertilization of soils with inorganic P fertilizers may therefore result in an environment that is not suitable to the formation of the symbiosis between host crops and native AMF populations.

Previous crop is also an important factor in the formation of AMF symbiosis by host crops. Pre-cropping with host crops provides the best environment for host crops to interact with AMF in field soils. Fallow and non-host crops decrease the population size of AMF communities and may select against certain species of AMF that potential host crops may most efficiently interact with. Proper management of crop rotations and fertilizer regimes seem to be the most important tools for exploitation of AMF symbiosis in the field.

When grown in a controlled environment, differences in the ability of historic and modern wheat cultivars were detected. Growth patterns for root and shoot production in historic cultivars are more conducive to successful exploitation of AMF symbiosis. Higher levels of root biomass production, relative to shoot biomass give the host plant a better opportunity to interact with AMF. Growth patterns of fungus also appear to be better suited to benefit by the host plant in historic cultivars. Fatty acid methyl ester analysis demonstrated larger proportional growth of the AMF hyphae externally in the soil in historic cultivars. This leads to increased opportunity for nutrient absorption and transfer to the host plant.

(c) TECHNICAL REPORT

Background:

Spring wheat (*Triticum aestivum* L.) is an important crop in agricultural production systems in Saskatchewan. In 1999 the acreage of spring wheat production was 12 735 000 acres, accounting for approximately 36% of all of the cropped acres in the province (Saskatchewan Agriculture and Food, 1999). Importance of wheat in Saskatchewan and western Canada has prompted crop researchers to develop improved varieties with increased yield potential and better end-use suitability. Western Canadian wheat breeding programs have similar objectives with respect to improved disease resistance, yield and grain quality. However, the programs may differ when focus is on development of varieties specifically suited to agro-climatic zones (DePauw, 1995). The end result is spring wheat cultivars specially suited to growth conditions resulting from regional climate and common agricultural practices. *Triticum aestivum* PI 167549 is a historical cultivar that originated in Turkey. *Triticum aestivum* cv. Red Fife is a historical spring wheat cultivar released in Canada ca.1845 that remained important until it was replaced by *T. aestevum* cv. Marquis ca.1910. *Triticum aestivum* cv. CDC Teal is a modern spring wheat cultivar (registered in 1991) that exhibits resistance to multiple diseases and is high yielding in the Parkland region of Saskatchewan (DePauw, 1995).

Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil microorganisms that form an obligate symbiotic association with most plants (Mosse, 1973a). The AMF association may increase or decrease plant growth potential depending on environmental conditions and the nature of the association itself. Generally, the AMF association is thought to increase plant growth potential by increasing water and nutrient uptake (especially phosphorus). Talukdar and Germida (1993) found that AMF communities exist in soils across four ecoregions of Saskatchewan. They also found that in all soils investigated, species composition of AMF communities differed and AMF readily colonized spring wheat (*Triticum aestivum* cv. Katepwa).

Agronomic practices can drastically affect the interaction of AMF with host crops. Incorporation of fallow and non-host crops into rotation decreases the inoculum

potential of the soil and may result in a significant reduction in the level of root colonization in future host crops. Cropping with host plants may also increase the inoculum potential of the soil, thereby increasing the level of root colonization in the following crop year. Proper crop rotation management is the most logical way for producers to promote the successful interaction of indigenous AMF with host crops.

Effect of phosphorous (P) on the mycorrhizal association has been studied and found to affect the degree of benefit conferred to the host plant (Amijee *et al.*, 1993; Baon *et al.*, 1993). Hetrick *et al.* (1996) found that mycorrhizal responsiveness of ten wheat varieties decreased with increased P fertilization and that AMF responsiveness was an inherited trait rather than a response to individual fungi. Growing evidence suggests that modern spring wheat varieties demonstrate varied response to AMF colonization. For example, Xavier and Germida (1998) found that four spring wheat varieties had varied susceptibility to AMF colonization as well as dissimilar abilities to exploit the rhizosphere AMF community. Hetrick *et al.* (1992, 1993) demonstrate that plant genotype likely affects the ability of wheat plants to be colonized and respond positively to AMF symbiosis.

The objective of this research program was to determine if wheat breeding practices have reduced the ability of modern Canadian spring wheat cultivars to participate in, and respond to, mycorrhizal symbiosis. The relative dependencies of historic and modern wheat cultivars on AMF symbiosis were studied. Specifically, the wheat varieties *T. aestivum* cv. PI 167549, *T. aestivum* cv. Red Fife and *T. aestivum* cv. CDC Teal were evaluated.

Three studies were conductedto address this objective. These studies comprised the M.Sc. research and thesis of Ms. Bobbi Hegalson and portions of that thesis are reported here with permission.

Study 1 – Interaction of Indigenous AMF with Field Grown Historical and Modern Spring Wheat Cultivars

Materials and Methods

Field Characteristics

In 1998 and 1999, field tests were set up at the Crop Development Centre (CDC) in Saskatoon at the Seed Farm (SF) and North Seed Farm (NSF) research sites. Three wheat varieties (PI 167549, Red Fife and CDC Teal) representing historical and modern wheat varieties were planted at each site.

In 1998, the SF and NSF sites were planted at early (April 28) and late (May 25) seeding dates, respectively. In 1999, SF and NSF sites were again planted at early and late seeding dates and replicate (n=6) soil cores (0-6") were collected from each plot on May 29, 1999. Samples were bulked and tested for chemical and physical properties at Envirotest Laboratories (Saskatoon, SK). Soil characteristics, seeding and sampling dates and cropping histories for the SF and NSF sites are listed in Table 1.

Sample Collection

Field plots were sampled at the flag leaf physiological stage of the wheat. Eight plants were sampled from each plot using a trowel. Plant shoots, roots and rhizosphere soil were collected and placed in 2lb plastic bags. In 1998 and 1999 shoot material was discarded and roots were removed from the rhizosphere soil and frozen at -20°C until further use. In 1999, soil and roots were sub-sampled and frozen for fatty acid methyl ester (FAME) analysis.

Most Probable Number of AMF Propagules in 1999 Field Soils

Soil samples were collected in May of 1999 from both SF and NSF field sites. A sub-sample was stored at 4°C and used as inoculum. A second sub-sample was ground and stored at room temperature until use as diluent soil.

Table 1. Cropping history (1997-1999) and nutrient status of CDC Seed Farm and North Seed farm experimental plots in 1999 (0-6' depth).

Location[a]	Cropping history	Cultivar[c]	Soil[d]					
			Texture	pH	NO_3-N (mg/kg)	P (mg/kg)[e]	K (mg/kg)	SO_4-S (mg/kg)
CDC Seed Farm	F-C-W	PI 167 549	Clay Loam	8.1	22	23	220+	10
		Red Fife	Clay Loam	8.1	21	22	220+	9
		CDC Teal	Clay Loam	8.2	23	21	220+	10
CDC North Seed Farm	B-BPD-W	PI 167 549	Loam	7.1	14	30+	300+	8
		Red Fife	Loam	7.1	11	30+	300+	9
		CDC Teal	Loam	7.1	9	30+	300+	5

[a]Seed Farm and North Seed Farm sites were seeded on April 28 and May 31 and plants were sampled on June 29 and July 14, 1999, respectively.

[b]Cropping history symbols are as follows: F:fallow; C:canola; W:wheat; B:barley; BPD:barley plough down.

[c]Cultivar represents wheat plants grown on wheat experimental plots in 1999.

[d]Soil nutrient testing was performed at Envirotest Laboratories in Saskatoon, Sk.

[e]Soil phosphorus represents Olson P.

The MPN assay was conducted according to a modified method of Porter (1979). Inoculum soil was added at a rate of 90.0g dry weight (DW) into 900.0g (DW) sterile 1:1 sand/soil mix in a 1000mL Nalgene bottle. The mixture was shaken 100 times, creating a 1/10 dilution of the field soil AMF inoculum. The dilution process was repeated to a final dilution of 10^{-5}. From dilutions 10^{-1}-10^{-5}, 165.0g of soil was added to each of five replicate Cone-Tainers.

Planting and Maintenance

Field pea seeds were surface sterilized for 2 minutes in 70% ethanol, followed by 4 minutes in 5% hyperchlorite solution. Using a flame sterilized metal probe (0.5 cm diameter), two holes were made in each tube (ca. 3.5 cm deep). Two surface sterilized pea seeds were aseptically transferred to each tube and covered with soil. Five days after germination, plants were thinned to one plant per tube and maintained in a growth chamber with conditions as follows: 16 hour days (25°C at a light intensity of 350-400 m^2s^{-1}) and 8 hour nights. Soil moisture was maintained at 60% of soil moisture holding capacity. The soil was amended with a modified Hoaglands macronutrient solution and 1/3 recommended rate of micronutrients (Talukdar and Germida, 1993).

Analysis of AMF Infection in Host Plants

Plants were harvested at 42 days after planting (DAP) and the roots separated from the growth medium. Roots were stained and cleared and analysed for AMF using the method of Koske and Gemma (1989). Roots were examined using a stereomicroscope until presence or absence of AMF infection was confirmed. The most probable number of infective AMF propagules and 95% confidence limits were calculated using the probability tables of DeMan (1975).

Spore Enumeration

In 1998, spore populations at the North Seed Farm field site were evaluated. AMF spores were extracted from rhizosphere soil using the wet sieving and decanting procedure (Gerdemann and Nicholson, 1963). Two 50 g soil sub-samples (<2mm) were extracted and spores separated from debris and residual soil using a sucrose density

gradient technique (Mertz *et al.*, 1979). Spores were vacuum filtered onto 45 mm diameter 0.8μm pore size filters (Millipore Corporation, Bedford, MA) and stored at 4°C until enumeration. A stereomicroscope was used to enumerate spores at magnifications of 40–60X. Photomicrographs of spore morphotypes were taken using an MC 100 stereomicroscope camera. Spore populations from the 1998 SF and 1999 SF and NSF sites were not evaluated.

Root Colonization

Frozen roots were thawed at room temperature before being rinsed thoroughly with distilled water to remove any adhering soil. Approximately 500 mg of roots were cut into 1.5-2.0 cm segments and ca. 250 mg placed into two replicate plastic perforated cassettes (Fisher Scientific, Nepean, ON). Roots were stained for AMF colonization as described above. The gridline-intersect method was used to estimate percent colonization (Giovannetti and Mosse, 1980).

Experimental Design and Statistical Analyses

Each treatment was replicated four times in a randomized complete block design. Spore enumeration and root colonization data were analysed using analysis of variance and LSD.

Results

Spore Populations Associated with Field Grown Historical and Modern Spring Wheat Cultivars

The total number of viable and non-viable AMF spores recovered from the NSF site in 1998 was significantly different between cultivars PI67549, Red Fife and CDC Teal (Table 2). Specifically, there were more spores associated with the rhizosphere soil of historical wheat lines. When spores were classified according to diameter, there were no significant difference in the total number of spores in a specific size class associated with the wheat cultivars.

Spore morphotypes collected from the rhizosphere soils at the 1998 NSF site were separated into two size classes: 212-106 μm and 105-35 μm. The number of spore

morphotypes in the two size classes ranged from 4 to 8 and was highest for PI 167549 (Photographs 1 and 2).

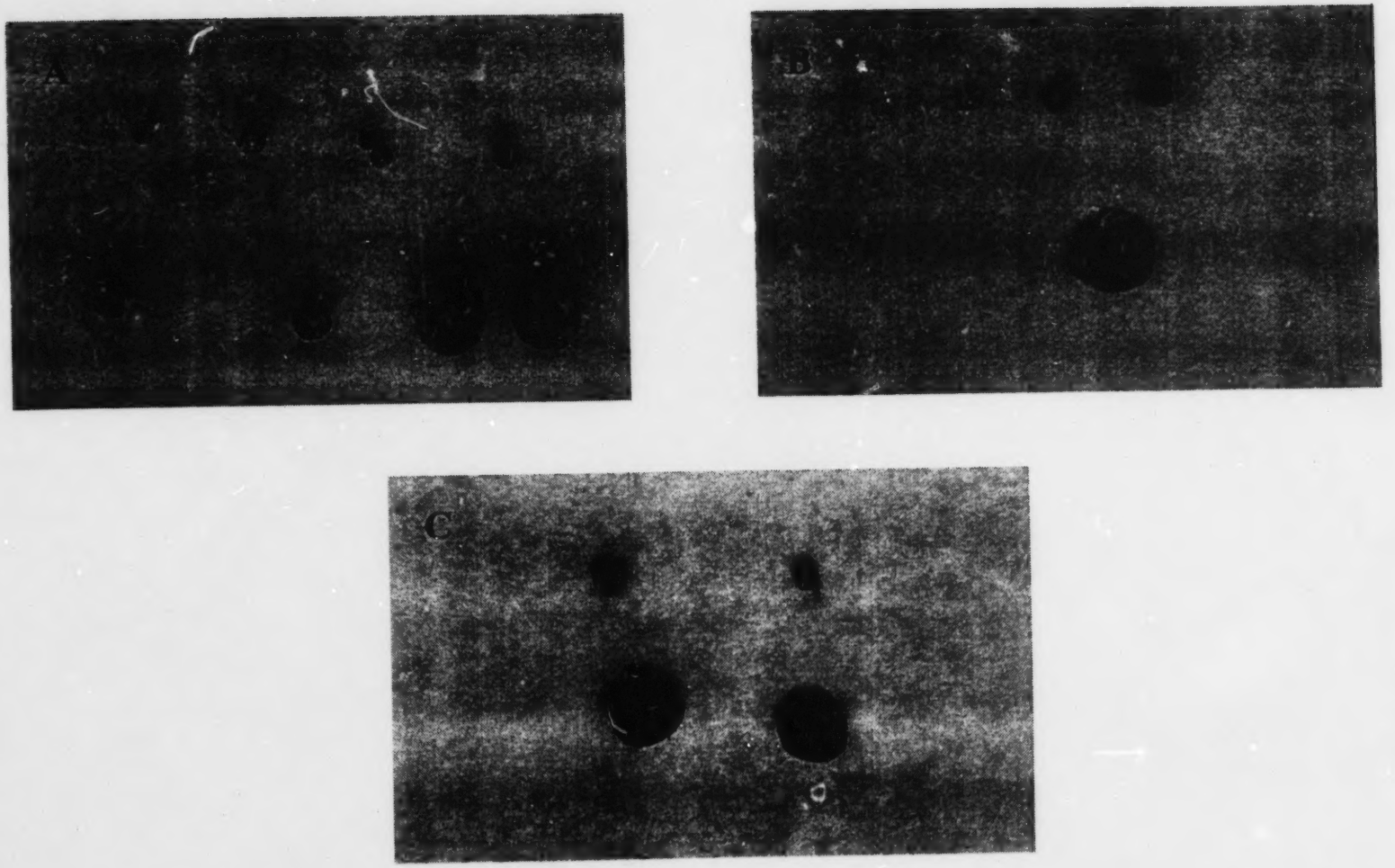
Root Colonization

AMF root colonization of modern and historical wheat cultivars was extremely low at both the Seed Farm and North Seed Farm field sites in 1998 and 1999. The very high levels of available P in both SF and NSF soils in 1998 and 1999 may have inhibited colonization of the wheat roots by indigenous AMF. Due to the extremely low levels of root colonization, it was difficult to compare the AMF root colonization of the three cultivars, however no significant differences between cultivars were detected.

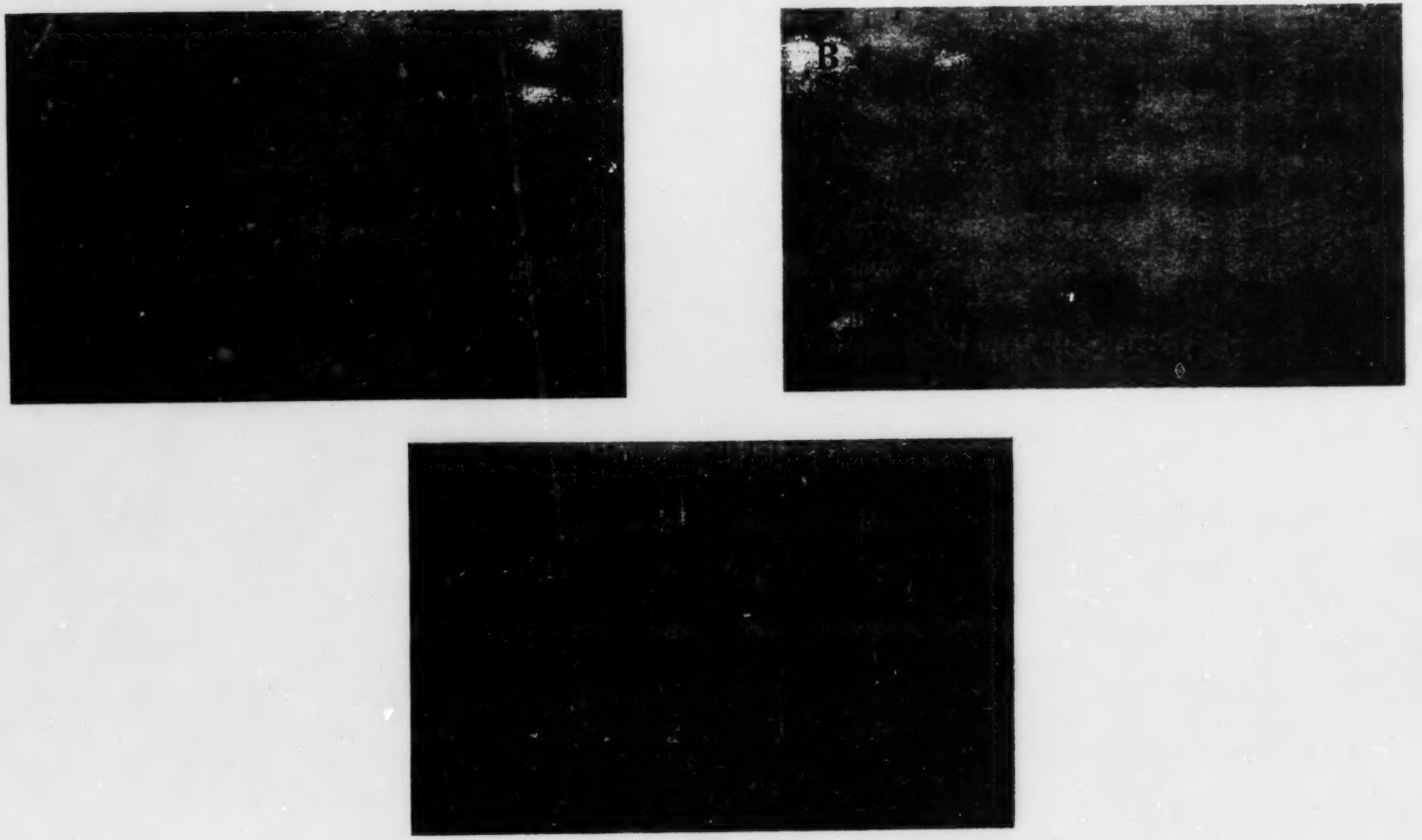
Table 2. Total number of viable and non-viable AMF spores extracted from the rhizosphere soil of modern and historic wheat cultivars at the 1998 NSF field site.

	Wheat cultivar		
# of spores extracted	PI 167 549	Red Fife	CDC Teal
Total	510 A	463 B	368 C
>212 µm	26	20	19
150-211µm	25	22	19
106-149µm	81	56	52
90-105µm	88	81	54
53-89µm	136	124	109
38-52µm	154	160	115

Numbers followed by different letters represent a significant difference at $p<0.05\%$



Photgraph.1. Arbuscular mycorrhizal spore morphotypes (212-106 µm diameter) extracted from 50 g of rhizosphere soil of PI 167549 (A), Red Fife (B) and CDC Teal (C) wheat cultivars at the 1998 North Seed Farm field site. Spores were separated into two distinct size classes using a wet decanting and sucrose density gradient extraction method. A stereomicroscope was used to examine spores at magnifications of 40–60X.



Photograph 2. Arbuscular mycorrhizal spore morphotypes (106-35 µm diameter) extracted from 50 g of rhizosphere soil of PI 167549 (A), Red Fife (B) and CDC Teal (C) wheat cultivars at the 1998 North Seed Farm field site. Spores were separated into two distinct size classes using a wet decanting and sucrose density gradient extraction method. A stereomicroscope was used to examine spores at magnifications of 40–60X.

Discussion

Evaluation of the AMF spore population in the 1998 Seed Farm soil demonstrated the presence of spores in sufficient numbers to induce root colonization in an agricultural field. However the indigenous AMF populations in the Seed Farm and North Seed Farm soils demonstrated little or no interaction with the three wheat cultivars.

The number of spores found in the Seed Farm soil is within a range that is typical of agricultural soils in Saskatchewan (Talkudar and Germida, 1993; Kucey and Paul, 1983). However, the presence of spores in soil does not necessarily mean that those spores will interact with potential host plants (Smith and Read, 1997). Arbuscular mycorrhizal spores can exist in soil in a dormant state, or in a non-viable state. The germination and growth of AMF in the rhizosphere can be dependent on many factors such as plant nutritional status (Pfeffer *et al.*, 1998) and plant symbiotic gene activity (Hirsh and Kapulnik, 1998). Therefore, although there were many AMF spores present in the 1998 and 1999 field soils, their germination and interaction with potential host plants cannot be assumed.

As wheat is known to interact with AMF in Saskatchewan soils (Kucey and Paul, 1983; Talukdar and Germida, 1993, 1994; Xavier and Germida, 1998) it is likely that the inhibition of the formation successful symbiosis in the 1998 and 1999 field trials is related to environmental conditions. Soil nutrient testing in the spring of 1999 revealed that both SF and NSF soils contained extremely high levels of available phosphorus. It is well known that high levels of soil available P generally inhibit the formation of AMF symbiosis with potential host plants.

Study 2 - Effect of Previous Crop on the Interaction of AMF with Field Grown Historical and Modern Spring Wheat

Materials and Methods

Soil Characteristics

Three field trials were set up at the Crop Development Centre Kernen Research Farm to study the effect of previous crop on the interaction of AMF with historical and modern wheat cultivars. The first trial was set up on a fallow soil, the second trial on canola stubble and the third trial on wheat stubble. The trials were seeded on May 24, 2000 and included the previously mentioned PI167549, Red Fife and CDC Teal cultivars. Soil samples (0-6") were collected from each of the three sites before seeding in May 2000 and analysed for nutrient status at EnviroTest Laboratories (Saskatoon, SK). Soil characteristics are listed in Table 3.

Parameter Analysis

Field plots were sampled at three intervals throughout the growing season to evaluate the development of the AMF symbiosis with time. Sampling dates were June 29, July 17 and August 21 (Photograph 3). Plants and rhizosphere soil were sampled as described in study 1 and stored until further analysis.

Mycorrhizal root colonization was analysed as described in Study 1. Plant shoot material was placed in a brown paper bag and dried for 72 hours at 65°C. Tissue samples were then ground to 20-mesh sieve size and stored until determination of shoot P content. Plant tissue digestion using a H_2SO_4-H_2O_2 mixture (Thomas *et al.* 1967) was used to determine P concentration.

Table 3. Characteristics of CDC Kernen fallow, canola stubble and wheat stubble field trial soils.

Previous Crop	Texture	pH	E.C.	NO_3-N (mg/kg)	P (mg/kg)[a]	K (mg/kg)	SO_4-S (mg/kg)
Fallow	Clay-loam	7.4	0.4	19	27	270+	11.5
Canola Stubble	Clay	7.8	0.4	10	25.5	255+	20.5
Wheat Stubble	Clay	7.9	0.4	11.5	25.5	255+	13

[a]Soil phosphorus represents Olson P.

Fatty Acid Methyl Ester Analysis of Soil and Root Material

Soil and root fatty acid methyl ester analysis was performed according the Cavigelli *et al.* (1995). Samples were analyzed using a Hewlett Packard 5890 Series II Gas Chromatograph fitted with a flame ionization detector and a capillary column (Hewlett-Packard Ultra II; 25 m by 200 µm; cross-linked 5% phenyl-methyl silicone with a film thickness of 0.33µm) using nitrogen as the carrier gas.

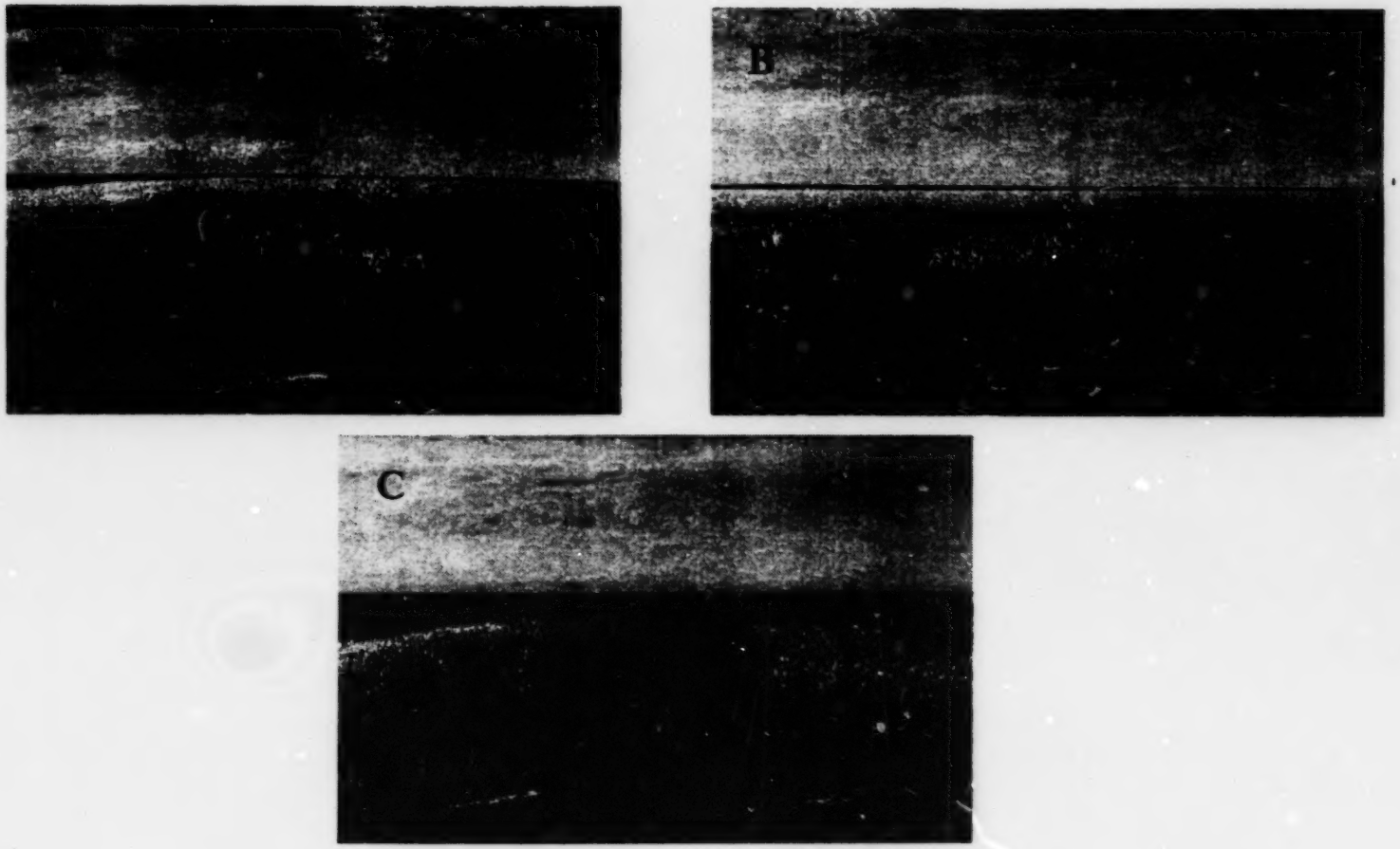
Fatty acid methyl ester analysis of root material was analyzed using the same procedure, however 0.6 g of root material is extracted. For comparative analysis, an adjusted response ratio of the mycorrhizal FAME biomarker 16:1ω5*cis* to the total fatty acid content of each sample was presented.

Most Probable Number

Most probable number of infective AMF propagules in each of the field soils was evaluated as described in Study 1.

Experimental Design and Statistical Analyses

Field plots in each trial were replicated four times in a randomized complete block design. Root colonization and shoot phosphorus content were analysed as described in Study 1.



Photgraph 3. Modern and historic wheat cultivars grown for 89 days on (A) wheat stubble soil, (B) fallow soil and (C) canola stubble soil.

Results

Most Probable Number of Indigenous AMF in Fallow, Canola and Wheat Stubble Soils

The most probable number of AMF infective propagules per gram of soil in the 2000 field soils were greater than 10^5 in the wheat stubble. The fallow soil contained $9.4x10^3$ infective propagules and $2.1x10^2$ were detected in the canola stubble.

AMF Root Colonization

AMF colonization of wheat roots in the field was affected by both sampling time and previous crop. However, there were no differences in the level of root colonization by indigenous AMF between the three cultivars (Table 4).

The effect of sampling time demonstrates a trend in which root colonization increased until 51 DAP and then did not change significantly by 89 DAP (Figure 1). Previous crop also affected AMF root colonization (Figure 2) and was highest on wheat stubble soil, intermediate on fallow and lowest on canola stubble soil. There was a significant interaction between time and previous crop on the AMF root colonization of field grown wheat. On wheat stubble soil, AMF root colonization was well established by the first sampling date (36 DAP) and increased until the second sampling time (51 DAP).

Table 4. AMF root colonization (n=12) of wheat grown on soil with three different cropping histories and sampled at three times during the growing season.

	AMF root colonization (%)		
	Sampling date		
	36 DAP	51 DAP	89 DAP
Wheat stubble	20 (27)[a] de[c]	41 (40) a	28 (32) bc
Fallow	11 (19) f	26 (31) cd	33 (36) ab
Canola stubble	6 (14) g	15 (23) ef	16 (24) e

$LSD_{0.001} = 9.4$[b]

[a] Numbers in parentheses represent arcsine*square root transformation of percentage data.

[b] LSD value is based on arcsine*square root transformed data.

[c] Means followed by different letters are significantly different at $p<0.001$.

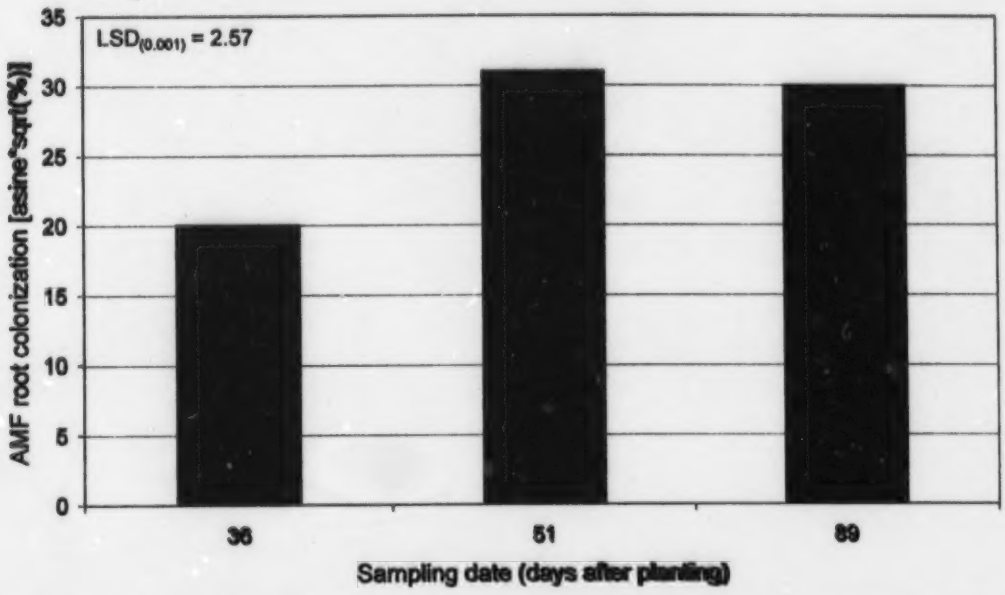


Figure 1. Effect of sampling time on AMF root colonization of modern and historical wheat cultivars grown in field soil containing indigenous AMF. Bars labeled with different letters represent means differing at $p<0.001$.

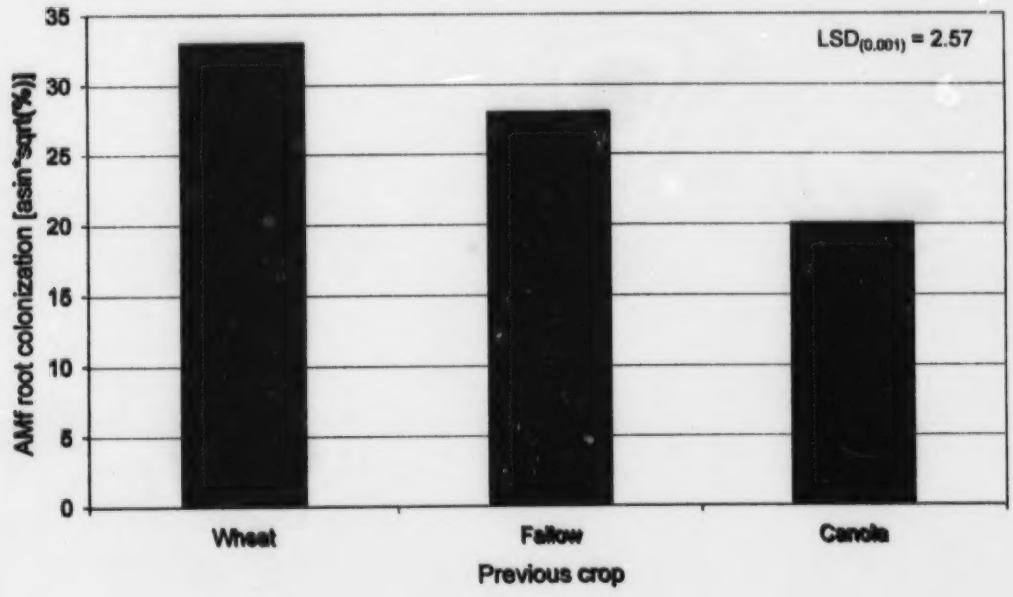


Figure 2. Effect of cropping history on AMF root colonization of modern and historical wheat cultivars grown in field soil containing indigenous AMF. Bars labeled with different letters represent means differing at $p<0.001$.

There was then a significant decrease in the level of AMF root colonization of wheat grown on wheat stubble between 51 and 89 DAP. On the fallow soil and canola stubble soils AMF root colonization was much lower at 36 DAP and increased substantially by 51 DAP. However, there was no significant increase in root colonization of wheat grown on these soils between 51 and 89 DAP. Wheat grown on canola stubble had extremely low levels of root colonization at 36 DAP and levels significantly increased over all sampling dates.

The adjusted response ratios (ARR) of the mycorrhizal FAME biomarker 16:1ω5*cis* to total fatty acid content of field soils were not significantly affected by any of the applied treatments. Adjusted response ratio of the 16:1ω5*cis* biomarker of root material however, was affected by previous crop, sampling time and cultivar. The ARR of wheat roots was highest at 51 DAP, followed by 89 DAP and lowest at 36 DAP. Adjusted response ratio was significantly higher in wheat roots grown on fallow soil, than in wheat grown on wheat and canola stubble (Figure 3). Wheat cultivar also affected the ARR of roots with Red Fife containing the highest proportion of the FAME biomarker. CDC Teal had a significantly lower ARR than Red Fife and PI 167549 was not significantly different from either of the other cultivars (Figure 4).

Plant Phosphorus Nutrition

Plant tissue P concentration of field grown wheat was significantly affected by previous crop, sampling time and cultivar. Phosphorus concentration of plant tissue was significantly higher at the 36 and 51 DAP sampling dates than at 89 DAP (Figure 5). Additionally, wheat grown on wheat and canola stubble had significantly higher phosphorus concentration that wheat grown on fallow soil (Figure 6). Phosphorus concentration of wheat tissue was also significantly higher in the modern CDC Teal cultivar than in the historical cultivars PI 167549 and Red Fife (Figure 7).

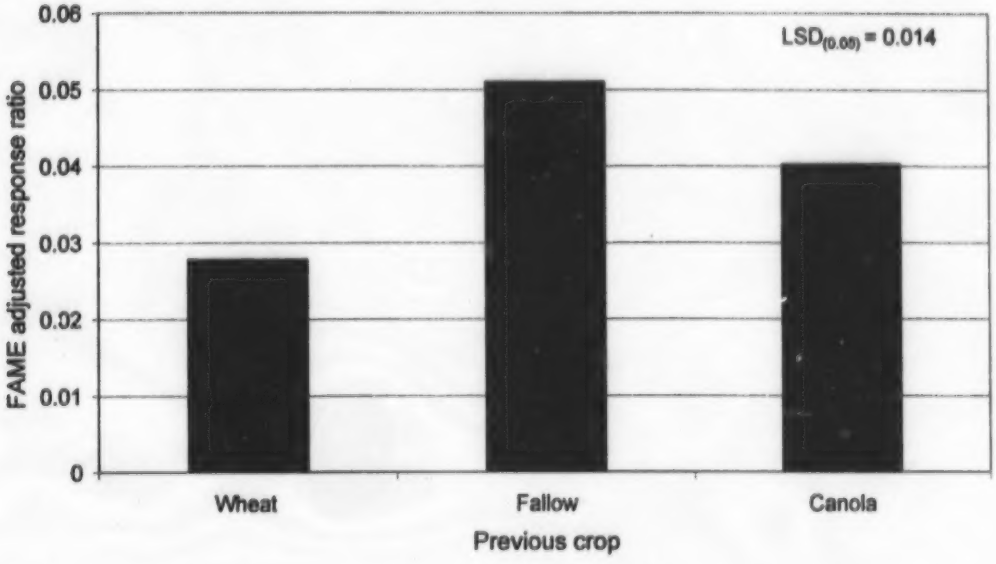


Figure 3. AMF FAME biomarker 16:1ω5*cis* in roots of field grown wheat grown on soils with different cropping histories. Bars labeled with different letters represent means differing at $p<0.05$.

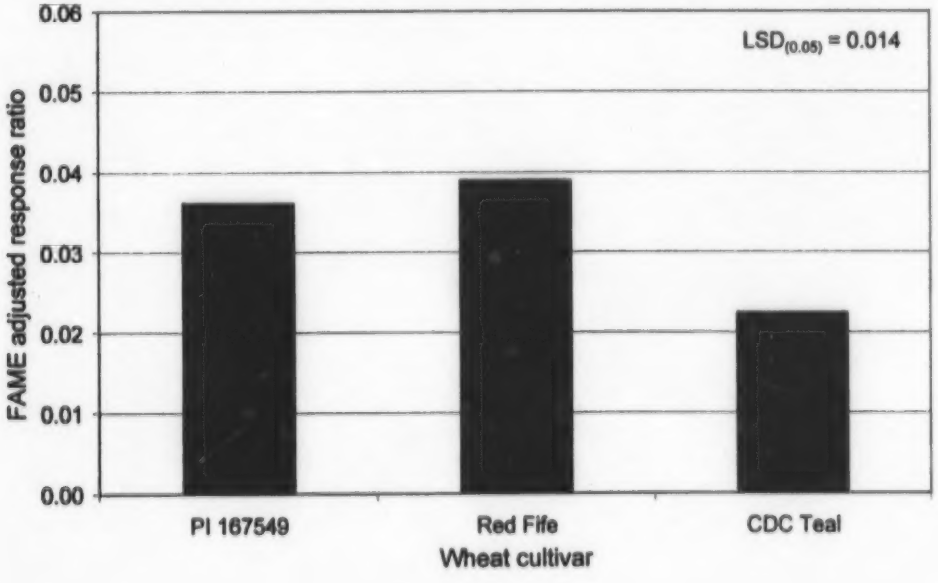


Figure 4. AMF FAME biomarker 16:1ω5*cis* in roots of modern and historic wheat cultivars. Bars labeled with different letters represent means differing at $p<0.05$.

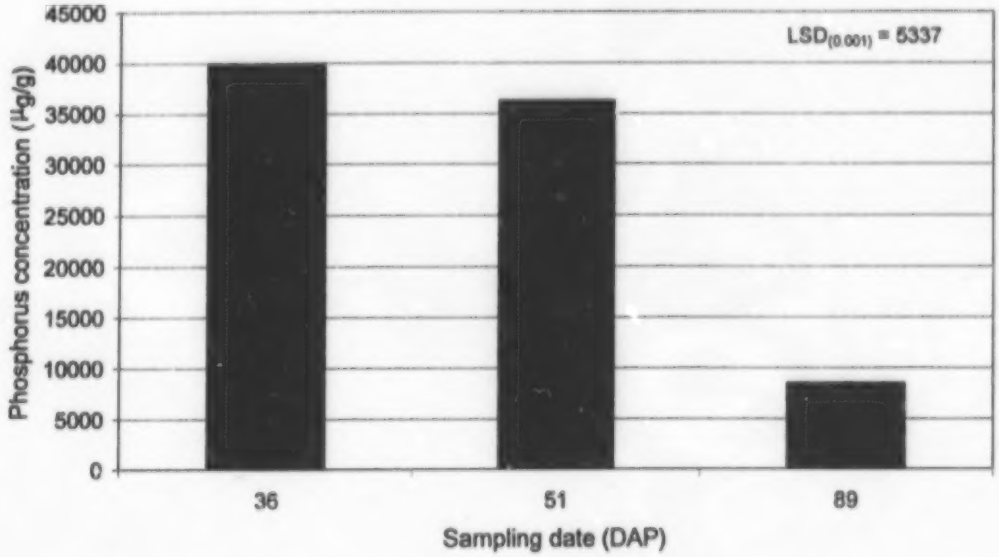


Figure 5. Influence of sampling time on phosphorus concentration of field grown wheat. Bars labeled with different letters represent means differing at $p<0.001$.

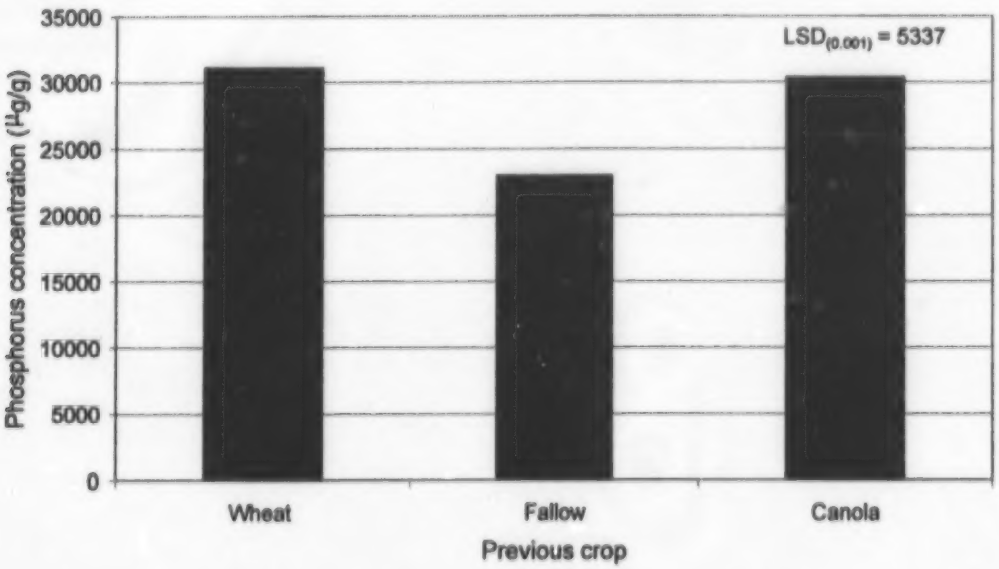


Figure 6. Influence of previous crop on phosphorus concentration of field grown wheat. Bars labeled with different letters represent means differing at $p<0.001$.

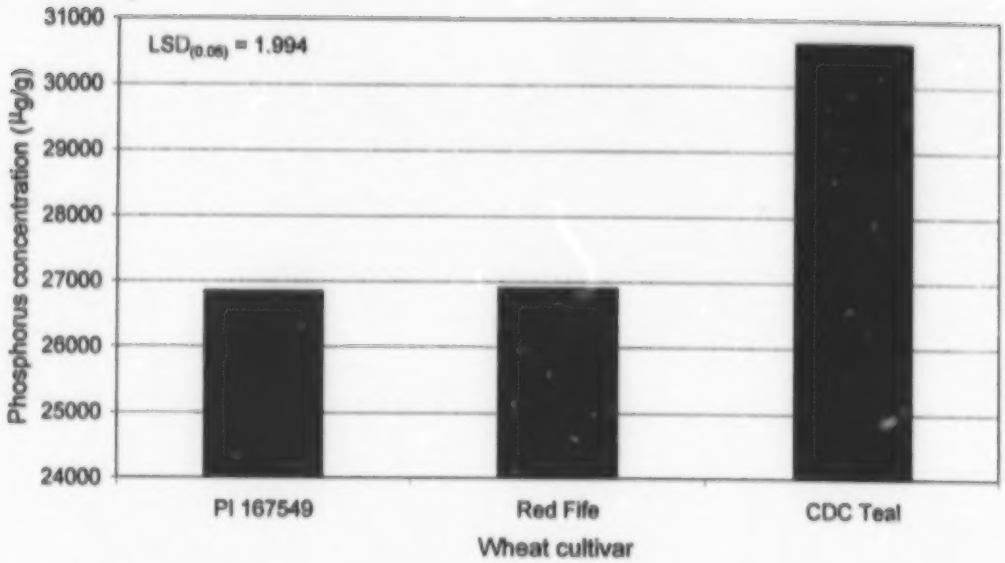


Figure 7. Phosphorus tissue concentration of modern and historic wheat cultivars grown in the field. Bars labeled with different letters represent means differing at $p<0.05$.

Discussion

Effectiveness of AMF symbiosis in promoting host plant growth and nutrition is related to the rate of development of the symbiosis. Early establishment of AMF symbiosis is important to the formation of a beneficial relationship with the host plant (Tinker, 1975) and is directly related to the inoculum density and inoculum potential of the AMF populations in soil. Kapulnik and Kushnir (1991) suggest that mechanisms affecting plant yield are established early in plant growth rather than at later stages of plant maturation. In addition, most of the macronutrient uptake by annual crops takes place early in the growing season, making early establishment of AMF especially important in those plants dependent on AMF for P nutrition.

The lack of differences in the AMF root colonization between the three cultivars suggests that under field conditions present in this study, the indigenous AMF communities in these soils were equally able to interact with modern and historic cultivars. Environmental factors that may have been important in this field experiment

include inoculum potential of the soil, species composition of AMF communities, soil nutrient status and destruction of AMF hyphal networks due to physical soil disturbance.

Inoculum potential of the soil dramatically affects the AMF colonization of plant roots (Wilson, 1984; Khan, 1988). In this study, cropping history significantly affected AMF root colonization of wheat. Colonization of wheat roots was highest on wheat stubble soil, intermediate on fallow soil and lowest on canola stubble soil. Levels of AMF root colonization in all three soils reflected the inoculum potentials of the soils before seeding. Results of the most probable number experiments conducted on these soils indicate that such reductions occurred in Saskatchewan field soils as the result of fallow and non-host crops. Our findings indicate that cropping to canola caused an even greater reduction than summerfallowing in the AMF inoculum potential of the soil.

Early establishment of AMF root colonization was much greater on wheat stubble soil than fallow and canola stubble soils. This may have important implications for the benefit of the host plant. Whether these differences were due solely to inoculum potential or are the result of changes in the species composition of AMF communities was difficult to determine.

Analysis of AMF FAME biomarker 16:1ω5*cis* in wheat roots of the modern and historic cultivars grown in the field revealed a significant effect of previous crop on the adjusted response ratio (ARR) of the AMF FAME biomarker. Interestingly, this parameter indicates that there was more mycorrhizal biomass within the roots of wheat grown on fallow soil than wheat grown on wheat and canola stubble soil. Significant differences in the presence of 16:1ω5*cis* within roots of the wheat cultivars grown in the field indicate that there was more fungal biomass within the roots of Red Fife than CDC Teal.

There were no differences in the AMF FAME biomarker ARR in the soil surrounding wheat roots in the field. Due to the heterogeneous nature of field soils, it is logical that the detection of statistically significant differences in the biomarker within soils is difficult and unlikely. A significant block effect indicated that there seemed to be a non-random distribution of AMF fungal biomass within these field soils. However, there was no block effect on any of the other parameters measured, including AMF root colonization or root FAME biomarker ARR. Therefore, it is unlikely that this

distribution of 16:1ω5*cis* within soil affected interaction of indigenous AMF with host plants.

Differences in the phosphorus concentration of shoot biomass of field grown wheat over time are likely due to the production of biomass. At 36 and 51 DAP, phosphorus concentration was high in shoot tissue. Plant P uptake occurs primarily early in the growing season and as shoot biomass production increases, a "dilution" effect occurs. Therefore, the significant decrease in shoot tissue P concentration between 51 and 89 DAP was likely due to the development of additional above ground biomass. Modern and historic cultivars had different shoot phosphorus concentrations. CDC Teal had significantly higher tissue P concentration, however the mechanism by which this increased uptake occurred is unknown. Previous crop also affected plant P uptake with lower shoot P concentration on the fallow soil, compared to wheat and canola stubble. However, evaluation of AMF root colonization and AMF FAME biomarker data does not support the theory that this increased tissue P concentration was due to mycorrhizal P uptake.

While there were no obvious trends that demonstrated that modern and historic cultivars interacted differently with indigenous AMF in Saskatchewan field soils, sampling time and cropping history clearly influenced the level of interaction of AMF with field grown wheat. Inclusion of non-host crops, fallowing and tillage operations are common practices in agricultural production that directly inhibit AMF symbiosis. Management of soil nutrient availability and crop rotations are the simplest methods for producers to exploit benefits from AMF symbiosis with common field crops, including wheat.

Study 3 – The Effect of Phosphorus Availability on the Interaction of Arbuscular Mycorrhizal Fungi with Historical and Modern Spring Wheat Cultivars

Materials and Methods

Field Characteristics

Soil was collected from a wheat stubble field in the Brown soil zone near Conquest, Saskatchewan (Land location) on May 29, 2000. The field soil was inherently low in available phosphorus and soil was collect from a knoll landscape position in order to obtain the topsoil that was lowest in organic matter and available nutrients. A representative sample was bulked, mixed 1:1 with silica sand and analysed for nutrient availability at Envirotest Laboratories, Saskatoon, SK. The soil characteristics are listed in Table 5. Phosphorus availability in a normal agricultural soil would be >30 mg/kg (60lb/ac) (Saskatchewan Agriculture and Food, 2001) and can therefore P levels in this soil can be considered inadequate for crop production.

Table 5. Soil characteristics of the Conquest field soil.

Soil Texture	pH	EC.	NO_3-N (mg/kg)	P (mg/kg)[a]	K (mg/kg)	SO_4-S (mg/kg)
Loam	8.5	0.5	6	6	300+	24+

[a]Soil phosphorus represents Olson P.

Most Probable Number of AMF in the Conquest Field Soil

Inoculum and diluent soils were sub-sampled from soil collected at Conquest on May 29, 2000. A bulk soil sample (<2 mm) was stored at 4°C for use as AMF inoculum. Most probable number of AMF propagules in the Conquest soil was determined as in Study 1.

Treatment Design

The phosphorus response study included one soil treatment, three plant treatments (i, ii, iii) and four phosphorus treatments (a,b,c,d). The plant treatments represent historical and modern spring wheat cultivars and the phosphorus treatments represent

low, intermediate and high levels of available phosphorus. Soil phosphorus levels indicated are the nutrient status of the soil after nutrient addition and mixing 1:1 with silica sand. Specifically, the treatments included:(i) PI 167549; (ii) Red Fife; (iii) CDC Teal: (a) 6 mg/kg KH_2PO_4; (b) 21 mg/kg KH_2PO_4; c) 36 mg/kg KH_2PO_4; (d) 51 mg/kg KH_2PO_4

Preparation and Maintenance of Soil and Wheat Cultivars

Field soil was ground prior to potting and mixed 1:1 (w/w) with sterile silica sand. Polyvinyl chloride (PVC) tubing (10 cm diameter) was cut into 35 cm lengths and used as "pots" to maximize rooting depth. Phosphorus was added to the soil mix as KH_2PO_4 in solution at rates of 0, 15, 30 and 45 mg/kg. The soil was inherently low in nitrogen (Table 5) and consequently, 25 mg/kg of nitrogen as NH_4NO_3 in solution to obtain recommended nitrogen availability (Envirotest Laboratories, Saskatoon, SK; 1999 Crop Production Manual). A total of 3.5 kg of appropriately fertilized sand/soil mix was added to each pot and stored in the growth chamber for 8 days to allow the soil to equilibrate before planting. Pre-germinated, surface-sterilized wheat seeds were planted at a depth of 3.5 cm and a rate of 5 seeds per pot and were thinned to two plants per pot following seedling establishment.

Plants were grown for 16 hour days (25°C at a light intensity of 350-400 m^2s^{-1}) and 8 hour nights. Soil moisture was maintained at 60% of moisture holding capacity and fertilized with a modified (minus P) Hoaglands macronutrient solution and 1/3 recommended rate of micronutrients (Talukdar and Germida, 1993).

Plant Response Parameters

Shoot and Root Biomass and AMF Root Colonization

Plants were harvested at 52 days after planting. Plant shoot material was cut off at the soil surface and dried at 65°C for 72 hours and shoot dry weights were measured. Roots were extracted from the soil and washed thoroughly with distilled water. Excess water was removed and root weight was measured. A root sub-sample was dried at 60°C for 48 hours to determine root moisture content. From these values total root dry weight was calculated. The remainder of the roots were placed in Whirlpack bags and frozen at -

20°c until further processing. A sub-sample of soil from each pot was frozen at -20°C for future FAME analysis. Mycorrhizal root colonization was analysed as described in Stuyd 1 and soil and root FAME analysis was performed as in Study 2.

Plant Phosphorus Dynamics

Dried shoot material was ground to <20-mesh and analysed for P using the method of Thomas *et al.* (1967). Total tissue phosphorus content was calculated using the tissue P concentration multiplied by the total above ground biomass. Specific P uptake was calculated by dividing the root dry weight by the total P uptake.

Experimental Design and Statistical Analyses

Each treatment was replicated four times in a completely random design in the growth chamber. Individual pots were randomized weekly to minimize position effects. Shoot biomass, root biomass, P uptake and specific P uptake data was Log_{10} transformed and analysed using analysis of variance (ANOVA) and least significant difference (LSD) using Minitab v.12 for Windows (Minitab Inc., 1998). Root colonization data was arcsine square root transformed before performing ANOVA and LSD.

Results

Most Probable Number of AMF Propagules in Conquest Soil

The most probable number of AMF infective propagules in the Conquest field soil was 1.70×10^2 per gram of soil.

Effect of Phosphorus and AMF on Shoot and Root Biomass of Wheat

Cultivar had a significant effect on the shoot biomass of modern and historical wheat. Differences in the maturity of the three cultivars at the harvest date were apparent. Red Fife and CDC Teal produced more shoot biomass than PI 167549, when averaged over all P levels (Figure 8). Similarly, there was a main effect of P level on the shoot biomass of wheat. While the addition of 15 mg/kg P significantly increased shoot biomass production, there seemed to be a threshold related to biomass production at this level of P availability as additions of 30 and 45 mg/kg P did not result in the production

of additional shoot material. There was no interaction of P level with cultivar for shoot biomass production.

Root biomass production was also significantly affected by cultivar, phosphorus availability and the interaction of the two main effects. Historic cultivars produced significantly more root biomass than the modern cultivar (Figure 9) and the addition of 15 mg/kg P caused a significant increase in root biomass production. However, additions of 30 and 45 mg/kg P did not result in additional root biomass. A significant effect of the interaction of cultivar and P level indicates that root biomass production by each of the cultivars is dependent on the specific level of available phosphorus (Table 6). The modern CDC Teal cultivar produced significantly lower root biomass than PI 167549 and Red Fife at the 0 mg/kg added P level. Both Red Fife and CDC Teal produced significantly lower root biomass than PI 167549 at the 15 mg/kg added P level. At the 30 mg/kg added P level, CDC Teal again produced significantly less root biomass than both Red Fife and PI 167549. At the highest level of P availability PI 167549 and Red Fife again produced significantly more root biomass than CDC Teal.

The response of PI 167549 to P resulted in a significant increase in root biomass production with the addition of 15 mg/kg P, but further addition of P did not increase root biomass production. Red Fife also responded to the addition of 15 mg/kg P and the further addition of P resulted in increased root biomass production. Increasing P availability also initiated a positive response in root biomass production by CDC Teal, however additions of P above the 15 mg/kg level did not produce additional root biomass.

Table 6. Mean (n=4) root biomass of modern and historic wheat varieties grown for 52 days in 3.5 kg of 1:1 sand/soil mix amended with 0, 15, 30 and 45 mg/kg P and containing indigenous AMF. Means followed by different letters are significantly different at $p<0.05$.

	Root biomass [Log_{10}(mg/pot)]			
	Added P level (mg P/kg soil)			
	0	15	30	45
PI 167549	2.83 cde	3.19 a	2.99 abc	3.06 a
Red Fife	2.71 e	2.93 bcd	3.06 a	3.11 a
CDC Teal	2.13 f	2.80 cde	2.76 de	2.95 bcd

$LSD_{(0.05)}$=0.199

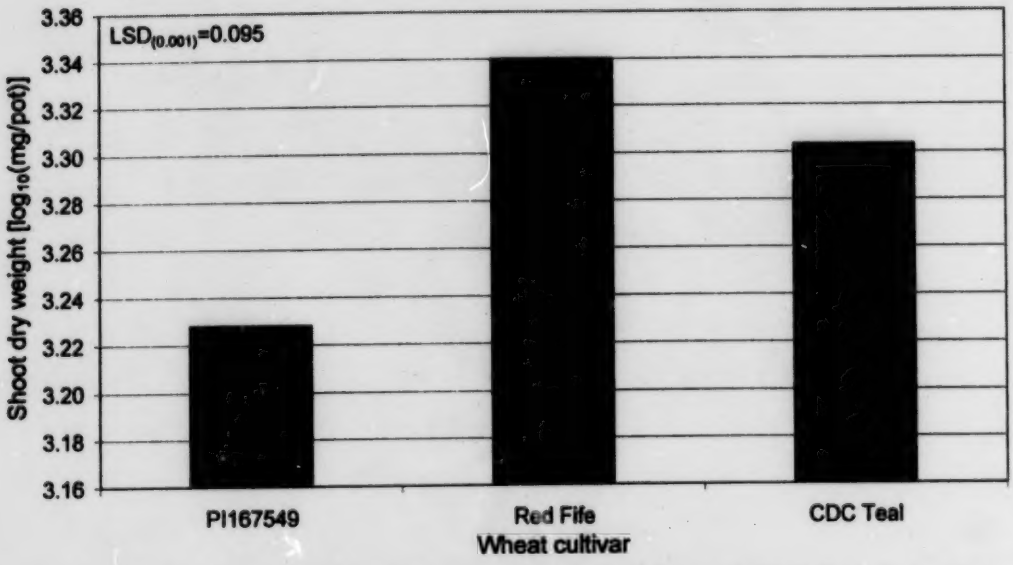


Figure 8. Mean (n=16) shoot biomass of PI 167549, Red Fife and CDC Teal grown for 52 days in 3.5 kg of 1:1 sand/soil mix containing indigenous AMF. Bars labeled with different letters represent means differing at $p<0.001$.

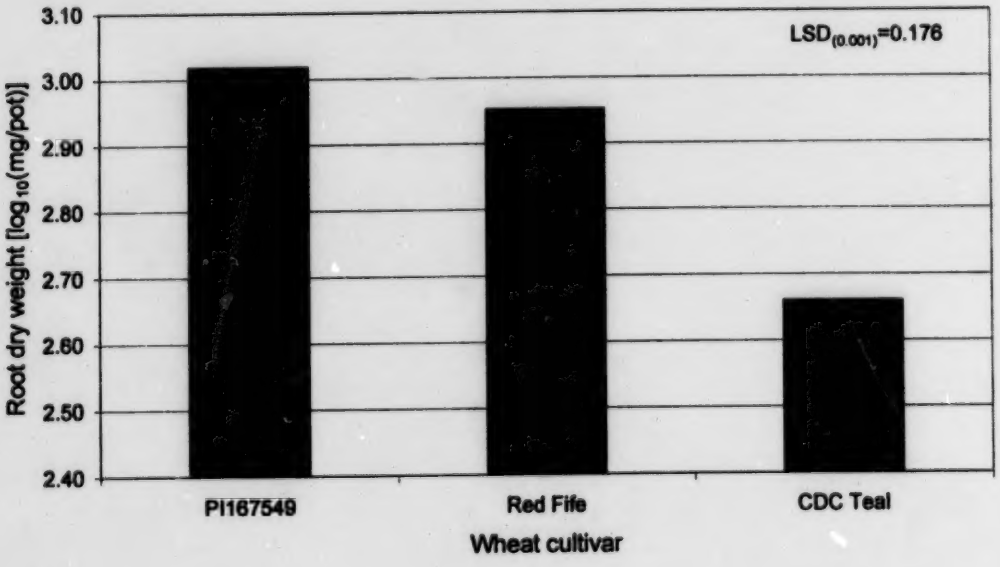


Figure 9. Mean (n=4) root biomass of PI 167549, Red Fife and CDC Teal grown for 52 days in 3.5 kg of 1:1 sand/soil mix containing indigenous AMF. Bars labeled with different letters represent means differing at $p<0.001$.

Effect of Phosphorus Availability and AMF on the Dynamics of Wheat Phosphorus Uptake

Total P uptake by modern and historical wheat cultivars at differing P availability was significantly affected by both cultivar and P level. CDC Teal had the greatest P uptake, followed by Red Fife and finally, PI 167549 (Figure 10). This trend indicates that modern cultivars take up more P, however it is important to note that there were substantial differences in plant maturity between the cultivars, with CDC Teal and Red Fife having reached a higher level of maturity.

A significant effect of P level was also apparent for P uptake responses). As P availability increased, P uptake also increased. However, the only significant difference in P uptake was found between the 0 and 15 mg/kg added P levels. This would indicate that plants seemed to reach a threshold with respect to P uptake at the 15 mg/kg level, at which point sufficient P was available for growth.

Specific P uptake of the three cultivars was significantly greater for CDC Teal than PI 167549 and Red Fife (Figure 11). The interaction between cultivar and P level was also significant for specific P uptake. Specific P uptake was highest for the CDC Teal cultivar at the 0 mg/kg added P level and lowest for the PI 167549 cultivar at the same P level. Within each cultivar, there was little change in the specific P uptake between the 15, 30 and 45 mg/kg added P levels (Table 7). However, differences between cultivars were apparent at different levels of P availability.

Table 7. Mean (n=4) specific phosphorus uptake of modern and historic wheat varieties grown for 52 days with indigenous AMF at 0, 15, 30 and 45 mg/kg added phosphorus. Means followed by different letters are significantly different at $p<0.05$.

	Specific P uptake [Log_{10}(µg P/g root)]			
	Added P level (mg P/kg soil)			
	0	15	30	45
PI 167549	4.14 g	4.36 fg	4.52 ef	4.47 ef
Red Fife	4.50 ef	4.81 cd	4.60 def	4.66 de
CDC Teal	5.23 a	5.09 abc	5.13 ab	4.87 bcd

$LSD_{(0.05)}$=0.286

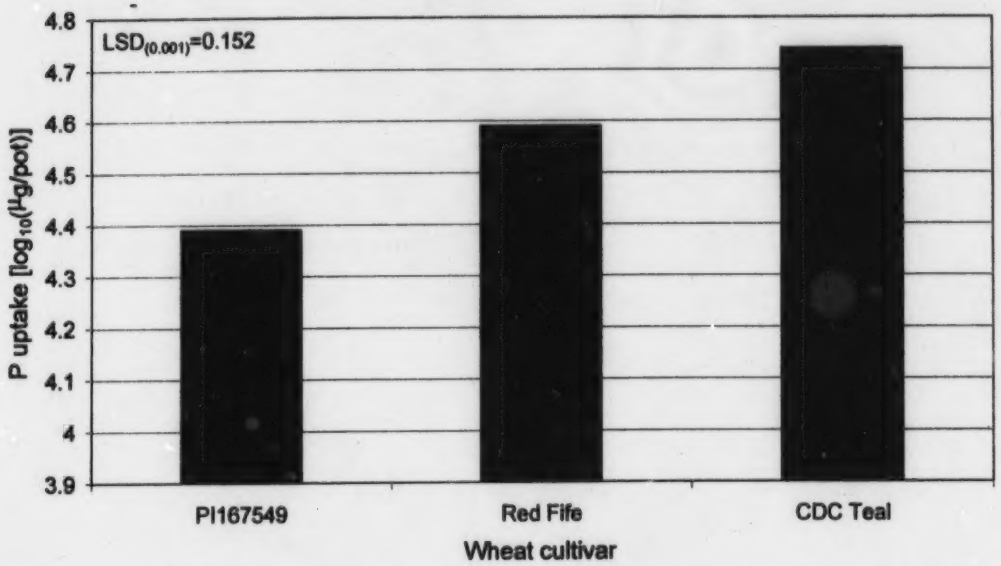


Figure 10. Total phosphorus uptake of modern and historic wheat cultivars grown for 52 days in 3.5 kg of 1:1 sand/soil mix containing indigenous AMF. Bars labeled with different letters represent means differing at $p<0.001$.

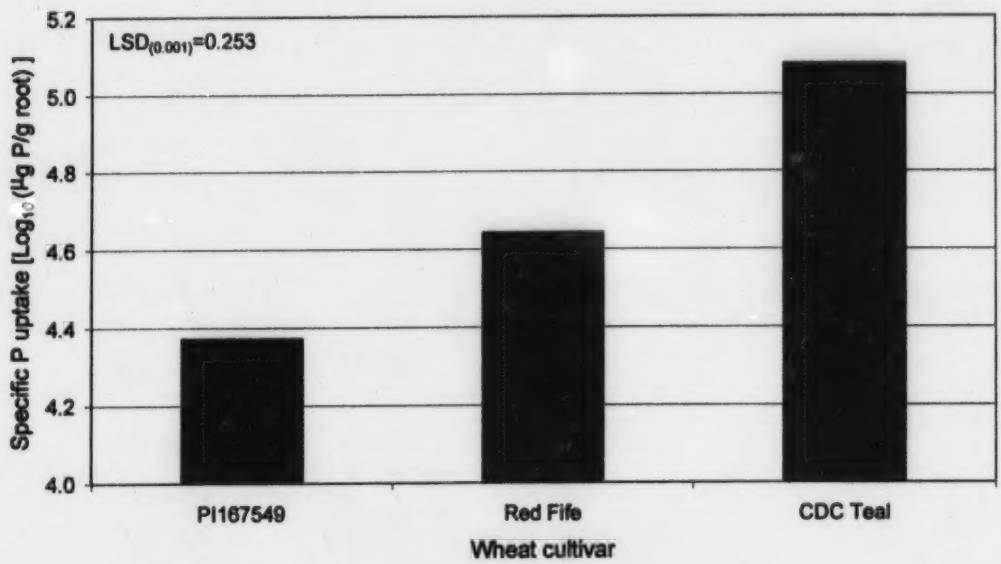


Figure 11. Specific phosphorus uptake of PI 167549, Red Fife and CDC Teal grown for 52 days in 3.5 kg of 1:1 sand/soil mix containing indigenous AMF. Bars labeled with different letters represent means differing at $p<0.001$.

Effect of Phosphorus on Mycorrhizal Colonization of Wheat Roots.

Arbuscular mycorrhizal root colonization of modern and historical wheat cultivars was significantly affected ($p<0.01$) by P level and the interaction of cultivar and P level. Root colonization was also affected ($p<0.07$) by cultivar. The modern CDC Teal cultivar had the highest level of % AMF colonized root, followed by Red Fife and PI 167549. The effect of phosphorus availability follows the previously established trend with plant biomass production where a significant difference ($p<0.001$) in AMF root colonization between the 0 and 15 mg/kg added P levels was noted. However, addition of P beyond 15 mg/kg did not affect AMF root colonization (Figure 12). A significant interaction effect of cultivar and P level indicates that root colonization of each of the cultivars responds differently to P, depending on the specific level of P availability (Table 8). The highest and lowest percent AMF root-length-colonized was found in the PI 167549 cultivar at 0 and 45 mg/kg added P levels, respectively. The historic PI 167549 has significantly higher root colonization than the modern CDC Teal cultivar at the 0 mg/kg added P level, however, at the 45 mg/kg added P level, this trend was reversed and CDC Teal had higher root colonization. Arbuscular mycorrhizal root colonization of Red Fife was not significantly different from either PI 167549 or CDC Teal with the exception of the 15 mg/kg level, when root colonization in Red Fife was significantly higher than CDC Teal.

Table 8. Mean (n=4) % AMF-colonized root length of modern and historic wheat varieties grown for 52 days with indigenous AMF at 0, 15, 30 and 45 mg/kg added phosphorus. Means followed by different letters are significantly different at $p<0.05$.

	% AMF-Colonized Root Length [asin*sqrt(%)]			
	Added P level (mg P/kg soil)			
	0	15	30	45
PI 167549	75.5 a	47.3 cd	32.1 fg	25.4 h
Red Fife	71.2 ab	44.7 d	33.0 ef	28.6 gh
CDC Teal	69.5 b	49.9 c	37.1 e	31.8 fg

$LSD_{(0.05)}$=4.78

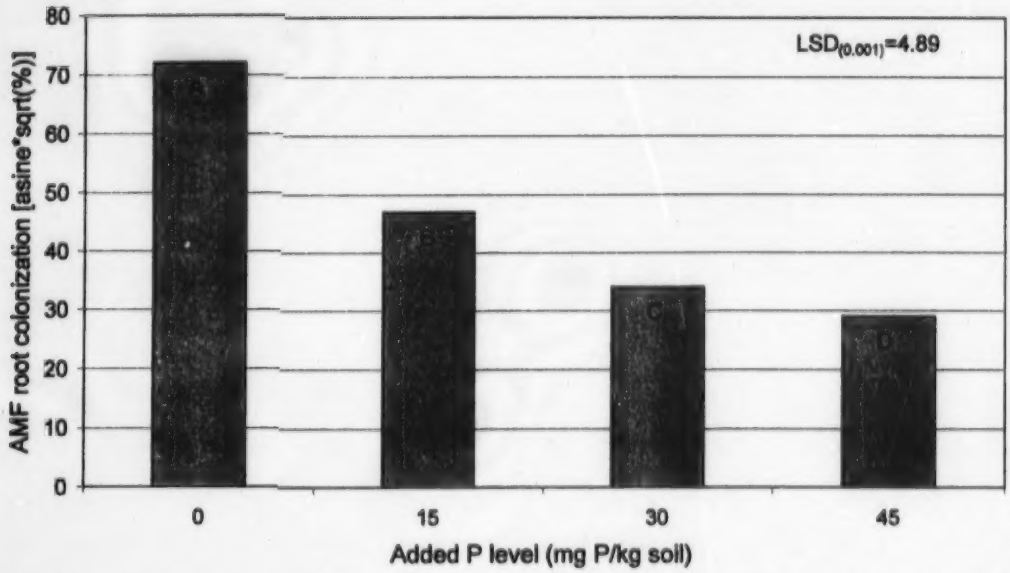


Figure 12. AMF root colonization of wheat grown for 52 days in 3.5 kg of 1:1 sand/soil mix amended with 0, 15, 30 and 45 mg/kg added P and containing indigenous AMF. Bars labeled with different letters represent means differing at $p<0.001$.

Analysis of AMF Fatty Acid Methyl Ester Biomarker 16:1ω5cis

Fatty acid methyl ester (FAME) analysis of root and soil material revealed differences between cultivars in the adjusted response ratio (ARR) of fatty acids in both the roots and the soil surrounding the roots. Cultivar significantly affected the ARR within wheat roots with CDC Teal and PI 167549 having substantially more FAME biomarker within their roots than Red Fife. In soil, the ARR of PI 167549 and Red Fife was significantly higher than CDC Teal, indicating the presence of significantly more FAME biomarker in the soil of these cultivars (Figure 13).

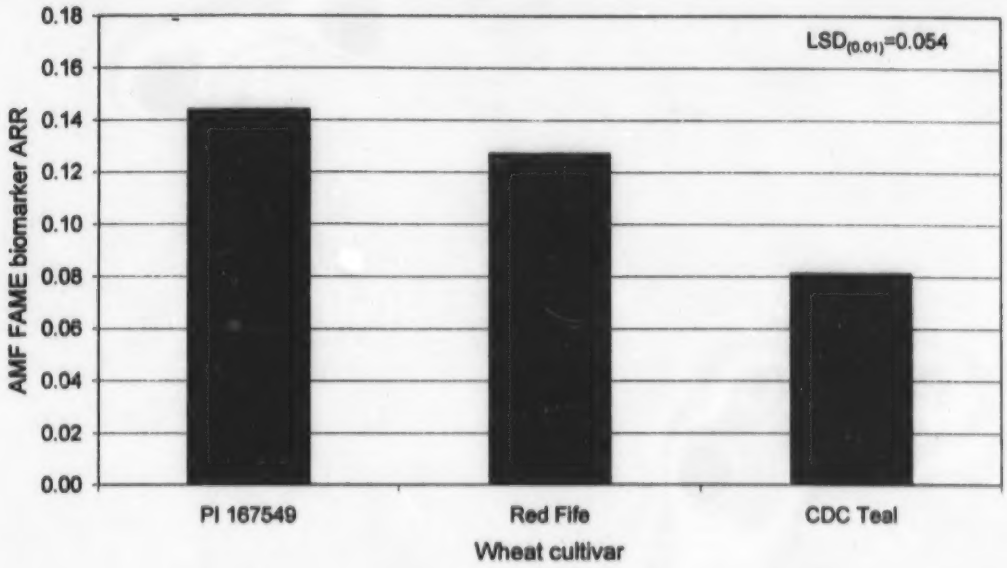


Figure 13. Adjusted response ratio (16:1ω5c/12:0) of AMF FAME biomarker 16:1ω5cis extracted from the soil surrounding the roots of modern and historic wheat cultivars grown for 52 days in 3.5 kg of 1:1 sand/soil mix amended with 0, 15, 30 and 45 mg/kg P and containing indigenous AMF. Bars labelled with different letters represent means differing at $p<0.01$.

Discussion

Spring wheat forms symbioses with arbuscular mycorrhizal fungal (AMF) communities in Saskatchewan field soils (Kucey and Paul, 1983; Talukdar and Germida, 1993) and with isolates from these communities under controlled experimental conditions (Talukdar and Germida, 1994; Xavier and Germida, 1998). The degree of benefit conferred to the host plant through the symbiosis can be highly variable, and may depend on various environmental factors including phosphorus (P) content of the soil, plant tissue P concentration (Hetrick *et al.*, 1996) and host plant genotype (Kapulnik and Kushnir, 1991).

Significant ($p<0.10$) differences in the level of AMF root colonization between cultivars in this study demonstrated that the modern CDC Teal variety had a higher level of root colonization than the historic cultivars, when averaged over all P levels. However, the degree of benefit that the host receives from the AMF symbiosis is not necessarily proportional to the level of root colonization. Because plant genotype may control phosphorus nutrition (Kapulnik and Kushnir, 1991; Hetrick *et al.*, 1993), the

presence of AMF in host roots and the resulting transfer of P to the host may not necessarily confer any benefit. Therefore, the presence of higher levels of AMF colonization in the roots of CDC Teal does not necessarily reflect a greater level of benefit derived by the modern cultivar from the symbiosis.

In this experiment, the specific relationship of each cultivar at varied levels of P availability was more informative in assessing the abilities of the modern and historic varieties to interact with AMF. The significant interaction effect of cultivar and P level demonstrated that the AMF root colonization of each cultivar was dependent on the specific level of P availability. When P availability was low, the historic cultivars PI 167549 and Red Fife were more extensively colonized by AMF. However, in the presence of luxurious P, CDC Teal was more extensively colonized. The ability of historic cultivars to be colonized by AMF was therefore much more sensitive to phosphorus availability than that of CDC Teal. This increased sensitivity of the historic cultivars to available P indicated their enhanced dependence on AMF for P nutrition.

Levels of AMF root colonization in the historic cultivars indicated that their relationship with the symbionts was more conducive to increased plant benefit than that of the modern cultivar. Plant shoot and root biomass production indicated that at the 15, 30 and 45 mg/kg applied P levels, phosphorus availability was sufficient for maximum plant growth. Correspondingly, the historic cultivars were able to limit AMF colonization of their roots under these conditions. The modern cultivar however, was unable to limit interaction with the fungus to the same extent when P availability was high. This inability of the modern cultivar to prevent the formation of significant levels of root colonization in an environment where P is not limiting may result in a photosynthate drain on the host plant.

Historic cultivars produced significantly more root biomass than the modern cultivars at all levels of P availability. Due to the large amount of root biomass produced, the significantly lower level of AMF root colonization at the 45 mg/kg P level in the historic cultivars could have resulted from an exhaustion of the inoculum potential of AMF in the soil. This is unlikely however, as the AMF root colonization of PI 167549 decreased at the with the increasing P availability, while no additional root biomass production occurred.

Phosphorus uptake dynamics of the modern and historic cultivars were an important consideration in the evaluation of the interaction effect of P availability and cultivar on AMF root colonization. Baon *et al.* (1991) found that barley cultivars that differed in phosphorus efficiency demonstrated variable responses to AMF, dependent on the level of P supply. Specifically, when P supply was low, cultivars that were agronomically P efficient responded less to mycorrhizal symbiosis than cultivars that were inefficient. In this experiment, when specific P uptake (μg P uptake / g root) was examined, CDC Teal was able to take up much more P per unit of root biomass than the historic cultivars. As P availability increased, the specific P uptake of CDC Teal decreased and the specific P uptake of historic cultivars increased. This indicated a higher level of P efficiency uptake by the modern cultivar. This increase P efficiency is an important factor in the examination of AMF symbiosis with the modern cultivar. A P efficient cultivar would not necessarily need to rely on the AMF symbiosis to obtain P, even under low P availability.

The fatty acid biomarker 16:1ω5cis has been identified as a taxonomic biomarker for identification and quantification of AMF in soil and colonized plant roots (Graham *et al.*, 1995). Analysis of AMF FAME biomarker 16:1ω5*cis* in soil surrounding roots of PI 167549 and Red Fife indicated that there was more external fungal hyphal biomass produced by AMF in association with the historic cultivars. External hyphae exploit soil in a more thorough manner than roots, are important for the increased uptake of P from soil, and thus vital to improvement of plant growth due to symbiosis (Johnson, 1993).. The presence of high levels of AMF FAME biomarker in the soil indicated that the interaction between the historic culitvars and the fungal symbiont was more likely to result in the successful increase of plant P content.

In this experiment, soil phosphorus availability significantly affected AMF root colonization with each increase in available soil P resulting in a decrease in the percent AMF root colonization of all cultivars. Inhibition of AMF root colonization in wheat roots by the presence of high levels of inorganic P has important consequences in agricultural production. Field trials conducted in 1998 and 1999 demonstrated that high levels of available phosphorus in Saskatchewan field soils resulting from inorganic fertilizer application inhibited AMF root colonization of wheat. Decreased levels of

AMF root colonization prevent the potential for contribution of indigenous AMF to plant growth.

This study demonstrated that modern and historic cultivars interact differently with indigenous AMF in the soil used. Historic cultivars were more dependent on AMF for P nutrition and were better able to inhibit the formation of AMF symbiosis when P was not limiting. The interaction of the modern cultivar with indigenous AMF was not as sensitive to P availability and the symbiosis seemed to be maintained at a higher level in the presence of luxurious P. Because a non-mycorrhizal treatment was not included in this study, it was difficult to determine the precise effects of the AMF symbiosis on plant growth. Further examination of possible effects of AMF symbiosis on carbon allocation at high and low P availability would be useful in determining whether plant growth of the cultivars was in fact increased by AMF symbiosis when P was limiting. Similarly, it would be useful to determine whether association of the modern cultivar at high P availability contributes to a carbon drain on the host, or whether the symbiosis has a purpose other.

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Publications:

Thesis

B. L. Helgason, M.Sc. The Interaction of Arbuscular Mycorrhizal Fungi in Saskatchewan Soils with Modern and Historical Wheat Cultivars. 2002

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B. L. Helgason, P. Hucl and J.J. Germida. 2002. Association of arbuscular mycorrhizal fungi in Saskatchewan soils with modern and historical wheat cultivars. (in preparation).

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J.J. Germida, "The Friendly Fungus" – CBC Radio Interview, Saskatoon, July 3, 2001

J.J. Germida, "Scientists Explore Friendly Fungus" – by A. Ewins, Western Producer, June 14, 2001

(d) Personnel:

Personnel:

		Percentage of time involved in project	Percentage of salary from ADF grant
Project director	J.J. Germida	20%	Nil
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(e) Equipment: None purchased

(f) Project Development Materials: No special material developed

(g) Project Photos: See report

(h) Acknowledgement: All publications and scientific posters and communications included an acknowledgement of support by Saskatchewan Agriculture and Food – Agriculture Development Fund.